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The Effect of the Addition of Fragrant Citronella Oil and Rhodinol to the handsanitizer on the Antibacterial Power of Staphylococcus aureus

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ABSTRAK

Hand sanitizer is an alternative to washing hands, it can also be antibacterial. Citronella and rhodinol oils consists of monoterpene compounds with major components in the form of aldehydes and alcohol. The oils have antibacterial properties. This study aims to increase the antibacterial power of the hand sanitizer by adding citronella and rhodinol. The experimental research method was carried out in a laboratory with two stages. The first stage was making hand sanitizers with variations in the addition of citronella and rhodinol. The second stage is to test the antibacterial power of the products. The addition of citronella oil and rhodinol can increase the inhibition zone against Staphylococcus aureus bacteria. The addition of rhodinol as an additive to the hand sanitizer gave a greater inhibition zone effect than the addition of citronella oil. The good result is a hand sanitizer made from 96% alcohol with 1% rhodinol additive.

KEYWORDS

Handsaitizer; citronella oil; rhodinol; antibacterial

INTRODUCTION

Hand sanitizer is one of the most desirable health equipment by today's society. The need for hand sanitizers is now increasing dramatically due to current global conditions [1]. Hand sanitizer, known as a hand antiseptic, is an alternative to washing hands with soap and water and can also be antibacterial. Hand sanitizer can be used as a disinfectant. The main ingredients of this hand sanitizer are isopropyl alcohol (Isopropanol), ethanol-propanol or povidone-iodine and triclosan [2]. When used excessively, hand sanitizers based on synthetic chemicals will cause skin irritation [3].

Essential oils, also known as ethereal oils, or volatile oils are commodity natural extracts from plant species. The essential oil that is produced today is citronella oil. According to research conducted by Eden, et al [4] citronella oil has 31 compounds with the main components being cis-geraniol (34.27%), β-citronellal (21.59%), and β-citronellol (7.43%). Citronella oil has the ability to inhibit bacteria Streptococcus mutans [5], Propionibacterium acnes [6], and others. Staphylococcus aureus is a human pathogen that causes a variety of infectious conditions in both nosocomial and community settings. Disinfectans are able to decolonize the Staphylococcus aureus [7].

The unstable price of crude essential oil causes low income of essential farmers. The crude citronella oil price ranges from Rp. 120,000 to Rp. 140,000 / kg in 2002, while in 2018 the market price of citronella oil was between Rp. 215,000-Rp. 225,000 / kg [7]. The application of a product for essential oils is necessary to increase the economic value of essential oils, especially citronella oil. There have been many researches on the manufacture of products using essential oils, one of which is the manufacture of deodorants made from

citronella oil [8]. Another study used fragrant citronella oil as a base for mosquito repellent skin lotion [9]. There is also research that makes hand sanitizers from celery essential oil [10].

In previous research, the manufacture of hand sanitizers had never been carried out using citronella oil and rhodinol as additives. The purpose of this study was to utilize citronella oil and rhodinol as antibacterial agents in hansanitizer products. Fragrant citronella oil and rhodinol are expected to be able to increase the antibacterial power of handsanitizer products.

MATERIALS AND METHODS

This research was conducted by carrying out several stages, the first is making a handsanitizer, making working cultures (Staphylococcus aureus), and antibacterial testing.

The materials used in this study were Staphylococcus aureus from the Microbiology Laboratory, Faculty of Medicine, Universitas Brawijaya. Staphylococcus aureus was inoculated in an oblique nutrient agar (NA) medium (test tube), using the zigzag pattern scratch method under aseptic conditions then anaerobic incubation at 37 ± 2°C for 24 ± 1 hour in Incubators. The active ingredient is used as an antibacterial in handsanitizer is citronella oil and rhodinol purchased at Institut Atsiri, Universitas Brawijaya. The changing variables in this study were the concentration of citronella oil and rhodinol added to the handsanitizer. Research variations can be seen in table 1.

Preparation of Hand Sanitizer

Preparation of 100 mL hand sanitizer using alcohol 96% (hand sanitizer A) and 80% (hand sanitizer B). A total of 83.3 ml of alcohol is put into a three neck flask. 4.17 mL of hydrogen peroxide are added and 1.45 mL of glycerol are added [11]. Citronella oil (or rhodinol) additive is added as much as 2% (changes according to variables). Aquades is added until it reaches a volume of 100 mL. Furthermore, the mixture is stirred until it becomes homogeneous at room temperature. Measurement of alcohol content in a hand sanitizer uses an alcohol meter. The measurement procedure is by dipping an alcohol meter into 100 mL of hand sanitizer, then the alcohol content value is obtained.

Table 1. Research variations

Concentration of Aditif	Variation	
	***Hand sanitizer* A**	***Hand sanitizer* B**
Without Addition	A 0%	B 0%
Citronella oil 1%	MSA 1%	MSB 1%
Citronella oil 2%	MSA 2%	MSB 2%
Rhodinol 1%	RHA 1%	RHB 1%
Rhodinol 2%	RHA 2%	RHB 2%

Preparation of Work Culture

Bacterial culture in the form of Staphylococcus aureus was suspended in nutrient broth media. Then staphylococcus aureus was incubated on erlenmeyer and shaker at room conditions for 24 ± 1 hour anaerobically. The working culture in the form of a Staphylococcus aureus suspension was tested and compared its turbidity with a standard solution of 0.5 McFarland using a turbidimeter to show the same value. This shows that the number of staphylococcus aureus bacterial cell colonies is around 1.5 x 108 CFU / ml [12] . The 0.5 McFarland solution was tested using UV-Vis and had an absorbance of 0.08-0.1 at a wavelength of 625 nm [13]. After the turbidity of the working culture approaches the standard solution, the work culture is ready to be inoculated for use in bacterial testing.

Antibacterial Testing

Antibacterial test using the disk diffusion method [12]. Disc paper with a diameter of 10 mm is inserted into the hand sanitizer. Furthermore, the disc paper is drained from the hand sanitizer so it doesn't drip. Disc paper containing hand sanitizer was affixed to the surface of the petri dish NA media that had been inoculated with working culture. Then incubated anaerobically at 37 ± 2oC for 24 ± 1 hour. This procedure is carried out for the concentration of citronella oil and rhodinol in different hand

sanitizers. The resulting clear zone diameter will be measured using a caliper.

RESULTS AND DISCUSSION

Result of Hand Sanitizer Formulation

The manufacture of hand sanitizers follows established procedures. In addition to using a variety of additives, this study also tried to use a variation of the basic alcohol ingredients of 96% (hand sanitizer A) and 80% (hand sanitizer B). The variation of the formula that has been made, the alcohol content is measured, so that each alcohol content is obtained.

Table 2. Comparison of alcohol content

Variation of aditif	Alcohol content (%)
A 0%	82
MSA 1%	83
MSA 2%	82
RHA 1%	82
RHA 2%	83
B 0%	75
MSB 1%	75
MSB 2%	75
RHB 1%	75
RHB 2%	75

Table 2 shows that all formulas have decreased alcohol content from the initial alcohol content, namely 96% and 80%. However, all still meet the minimum alcohol content standard of more than 60%. A minimum alcohol content of 60% can still kill bacteria by denaturing microbes [14].

The performance of adding citronella oil additives

The performance of the antibacterial power of citronella oil and rhodinol is shown in the figure 1.

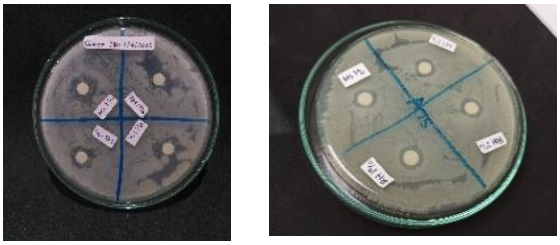


Figure 1. The performance of the antibacterial power

The addition of citronella oil additive to the hand sanitizer provides a greater antibacterial effect than using only alcohol as an antibacterial agent. This can be seen in the table 3.

Table 3. The diameter of the inhibition zone at various variations in the addition of citronella oil

Variation of aditif	Inhibition zone diameter (cm)		
	Trial 1	Trial 2	Average
A 0%	0.840	0.000	0.420
MSA 1%	0.940	1.300	1.120
MSA 2%	1.135	1.575	1.355
B 0%	0.000	0.000	0.000
MSB 1%	1.040	0.910	0.975
MSB 2%	1.185	1.040	1.113

From the data table 3, it can be seen that the more additives are added, the larger the diameter of the zone of inhibition against Staphylococcus aureus bacteria. The change in the increase in the inhibition zone can be shown in the figure 2.

Fragrant citronella oil is also an antibacterial agent that can kill Staphylococcus aureus bacteria, because citronella oil has major compounds in the form of aldehydes and alcohol, namely cis-geraniol (34,27%), β-citronellal (21,59%), dan β-citronellol (7,43%) [4].

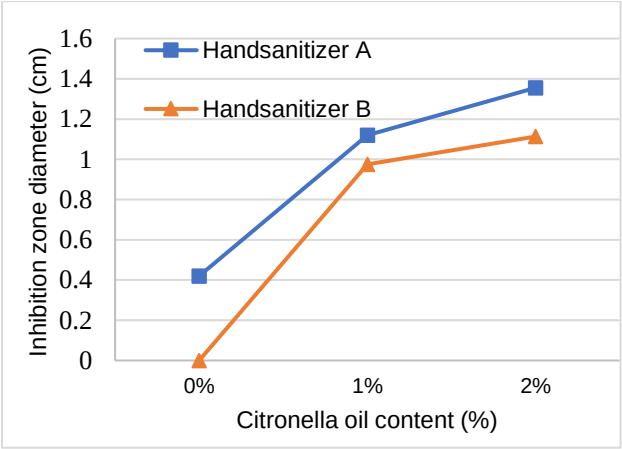
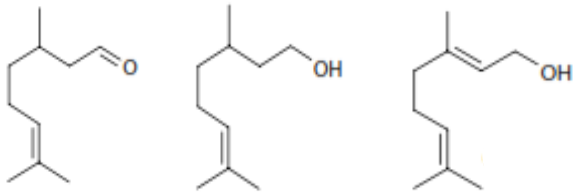


Figure 2. Changes in the diameter of the inhibition zone to the addition of citronella oil content

The alcohol group has an OH (hydroxyl) group which has antimicrobial activity and has large solubility in the cell membrane, while the aldehyde has a carbonyl group which has the same role as the hydroxyl group in alcohol [15].



(a) (b) (c)

Figure 3. Compounds (a) Citronellal, (b) Citronellol, (c) Geraniol [16]

Fragrant citronella oil has terpenoid compounds that can damage the cell wall structure and can interfere with the work in the cytoplasmic membrane of bacteria [17].

Table 4. The diameter of the inhibition zone at various variations of rhodinol addition

Variation of aditif	Inhibition zone diameter (cm)		
	Trial 1	Trial 2	Average
A 0%	0.840	0.000	0.420
RHA 1%	1.550	1.580	1.565
RHA 2%	1.240	1.740	1.490
B 0%	0.000	0.000	0.000
RHB 1%	1.100	1.090	1.095
RHB 2%	1.160	1.280	1.220

The performance of adding rhodinol additives

Rhodinol is a fraction of citronella oil. Rhodinol is a mixture of citronellol and geraniol fractions [4]. The antibacterial effect of hand sanitizers with the addition of rhodinol can be shown in the table 4.

Table 4 shows that the diameter of the inhibition zone when adding rhodinol is greater than before adding rhodinol, both from the formulation of hand sanitizer A and hand sanitizer B. Changes in the increase in the diameter of the inhibition zone can be seen in Figure 4.

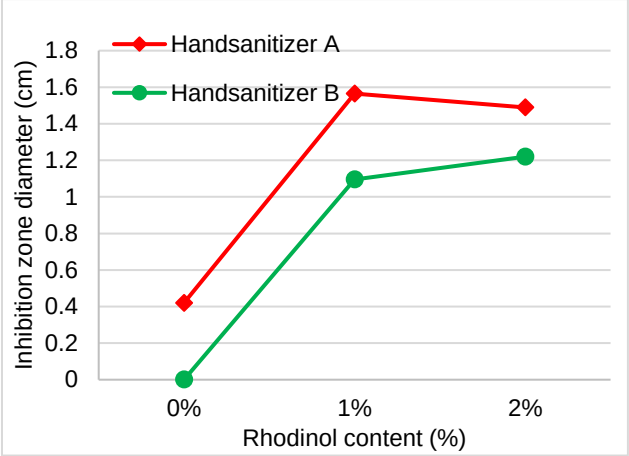


Figure 4. Changes in the diameter of the inhibition zone to the addition of rhodinol content

The addition of the hanbat zone against Staphylococcus aureus bacteria occurs because rhodinol is also an antibacterial compound. Rhodinol is a mixture of citronellol and geraniol, so that rhodinol also has an OH group in its compound composition [4].

CONCLUSIONS and SUGGESTION

The addition of citronella and rhodinol additives can increase the inhibition zone against Staphylococcus aureus bacteria. The addition of rhodinol as an additive to the hand sanitizer provided a greater inhibition zone effect than the addition of citronella oil as an additive. The biggest zone of inhibition is a hand sanitizer made from 96% alcohol with 1% rhodinol additive.

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